Genetic Diversity in Faba Bean (*Vicia faba* L.) Using Inter-Simple Sequence Repeat (ISSR) Markers and Protein Analysis

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Abstract: The present study aims to assess the genetic diversity among 10 varied faba bean cultivars, collected from two wide-ranging geographical locations of Egypt. Variability based on the DNA level was inspected through nine ISSR-PCR screening, which showed obvious differences among the various *Vicia faba (V. faba)* cvs. A total of 576 ISSR loci were detected and 398 (69.10%) of them were polymorphic, which represent a relatively high polymorphism level. Cluster analysis via ISSR markers separated three green large-seeded cvs. (*V. faba* var. major) from dry small-seeded cvs. (*V. faba* var. minor). The small-seeded cvs. were further classified into two sub-clusters according to two geographic locations. The first sub-cluster included dry small-seeded cvs. grown under clay soil conditions (Abies location). However, the second sub-cluster integrated the similar dry small-seeded cvs. but were grown under calcareous soil conditions (Fuka location). SDS-PAGE analysis of various faba bean leaf proteins reflected some variations among studied *V. faba* populations. The results clarified that ISSR markers and protein analysis were helpful to recognize genetic variation among faba bean cultivars.
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Key words: Cluster analysis, Egypt, faba bean, genetic diversity, ISSR markers, protein analysis.

1. Introduction

Faba bean, broad bean or field bean (*V. faba* L.; 2n = 12) is a major food and feed grain legume owing to the high nutritional value of its seeds, which are rich in protein 27-34% (**Link *et al*., 1995; Duc, 1997**). It is considered as one of the major sources of cheap protein and energy in Africa, parts of Asia and Latin America, where most people cannot afford meat sources of protein (**Duc, 1997; Alghamdi, 2009**). In Egypt, faba bean is among the main nutritional source of plant proteins (**El-Danasoury *et al*., 2008; Bakry *et al*., 2011**). Its consumption exceeded 440,000 t in 2001 (**FAO, 2002**). Nevertheless, the total production of this crop is still limited and falls to cover the increasing local consumption, so there is a prerequisite to enlarge the production by expansion throughout reclaimed areas which signify the scope of cultivated lands (**Khalafallah *et al*., 2008; Bakry *et al*., 2011**).

Nowadays, with increasing the number of faba bean varieties, it is difficult to differentiate these varieties based on morphological characters alone. These characters are either influenced by environmental factors and stage of plant development or reveal limited variation (**Terzopoulosa and Bebeli, 2008**). This has led to the progress of the new steady parameters like use of their genetic material (nucleic acids and proteins) as a tool for varietal identification (**Vishwanath *et al*., 2011**).

Recently, DNA-marker approaches have become gradually more utilized for taxonomic and phylogenetic analyses. They are not affected by environmental factors or by plant developmental stages. Besides, these approaches have potential for the routine testing of the genetic diversity and purity of accessions held in germplasm collections (**Gilbert *et al*., 1999**). The most commonly used polymerase chain reaction (PCR)-based marker systems for genetic diversity and relationships in faba bean species are randomly amplified polymorphic DNA (RAPD) (Link *et al*., 1995; El-Danasoury *et al*., 2008), amplified fragment length polymorphism (AFLP) (**Zeid *et al*., 2003; Duc *et al*., 2010)** and species specific repeats (SSR) (**Zeid *et al*., 2009**). The main limitations of these methods are low reproducibility of RAPD, high cost of AFLP and the necessity to know the flanking sequences to develop species specific primers for SSR polymorphism (**Belaj *et al*., 2003; Jabbarzadeh, *et al*., 2010**).

Inter-simple sequence repeat (ISSR-PCR) is a route that overcomes most of these technical limitations (**Reddy *et al*., 2002; Chen *et al*., 2008**). It

is a fast and simple system with a cost-efficient as well as it does not require any prior knowledge about the sequences to be amplified, being tremendously useful in genetic diversity, phylogeny, genomics and evolutionary studies (**Hu *et al*., 2003, Chen *et al*., 2008; Aguilera *et al*., 2011**). ISSR analysis has been successfully documented to determine genetic diversity and relationships in numerous economic legume species such as cow pea (**Ajebade *et al*., 2000**), common bean (**Galvan *et al*., 2003; Gonzales *et al*., 2005**), chickpea (**Sudupak, 2004**), in addition to faba bean (**Terzopoulosa and Bebeli, 2008**). The aims of this work is to: (i) identify and test ISSR-PCR markers for screening genetic diversity in the two commonly cultivated faba bean groups; dry small-seeded and green large-seeded cvs. (ii) Estimate the genetic diversity and relationships among these cvs. Under two varied geographic regions in Egypt rooted in ISSR markers and protein analysis.

2. Materials and Methods

Plant material:

Ten faba bean cvs. Collected from two different geographic locations; Abies, (Alexandria Governorate, 31° 12′ 46″ N and 29° 59′ 30″ E) and Fuka, (Matrouh Governorate, 31° 07′ 30″ N and 28° 05′ 00″ E) were used to ISSR-PCR detection markers. These cvs. represent the green large-seeded form (*V. faba* var. major), which are known as broad beans and the main vegetable type **(Rubatzky and Yamaguchi, 1997),** as well as the dry small-seeded types, (*V. faba* var. minor) which are known as field beans (Table 1).

DNA Extraction

Total DNA was extracted from 1 g of young leaves using Biospin plant genomic DNA extraction kit (Bioer Technology Co., Ltd. China). DNA quality was checked using 1.0% agarose gel electrophoresis and its concentration was determined spectrophotometrically.

ISSR-PCR

Nine random primers (Sigma, Germany) were selected for ISSR analysis (Table 2).

ISSR amplification was carried out using thermo-cycler (Eppendorf, Hamburg) in 25 µl of mixture containing 10 ng of genomic DNA, 10x Taq polymerase buffer, 50 mM $MgCl_2$, 0.2 mM each of dATP, dTTP, dCTP, dGTP, 25.0 p moles for each primer, and 0.5 U Taq DNA polymerase (Promega). Amplification conditions were: 95°C for 5 min; 45 cycles of 95°C for 1 min. 95°C for 1 min followed by annealing. The annealing temperature for PCR amplification was found to vary according to the base composition of the primers, for 1 min followed by a final extension 72°C for 10 min. Amplified products were fractionated by electrophoresis in 2% (w/v) agarose/TBE gels, visualized, and documented using a gel documentation and image analysis system (Alfa Imager M 1220, Documentation and Analysis System, Canada).

Table (1): Geographical distributions and species characteristics of the 10 faba bean cultivars.

Geographic areas	Cultivar name	Cultivar type	Seed type	Cultivar origin	Soil texture
Fuka, Matrouh					
1	ILB 450	Dry small-seeded	Minor	ICARDA	Calcareous
2	Misr I	Dry small-seeded	Minor	Egypt	Calcareous
3	Giza 843	Dry small-seeded	Minor	Egypt	Calcareous
4	Sakha 3	Dry small-seeded	Minor	Egypt	Calcareous
5	Rena Mora	Dry small-seeded	Minor	Spain	Calcareous
6	Giza 3	Dry small-seeded	Minor	Egypt	Calcareous
7	ILB 312	Dry small-seeded	Minor	ICARDA	Calcareous
8	Giza 716	Dry small-seeded	Minor	Egypt	Calcareous
Abies, Alexandria					
9	Aquadolce	Green large-seeded	Major	Spain	Clay
10	Aspany1	Green large-seeded	Major	Spain	Clay
11	Aspany2	Green large-seeded	Major	Spain	Clay
12	ILB 450	Dry small-seeded Dry small-seeded	Minor	ICARDA	Clay
13	Misr I	Dry small-seeded Dry small-seeded	Minor	Egypt	Clay
14	Giza 843	Dry small-seeded Dry small-seeded	Minor	Egypt	Clay
15	Sakha 3	Dry small-seeded	Minor	Egypt	Clay
16	Rena Mora		Minor	Spain	Clay
17	Giza 3		Minor	Egypt	Clay
18	ILB 312		Minor	ICARDA	Clay

Table (2): ISSR primers, their sequences, annealing temperature, size of amplified fragments (bp), total number of amplified fragments (TAF), number of polymorphic bands (PB), polymorphic percentage (PB%) and specific bands identified per primer used to access genetic diversity of 10 faba bean cultivars.

Primers	Sequence 5′→ 3′	Annealing temp. (°C)	Size range (bp)	TAF	PB	PB (%)
ISSR-1	GAGAGAGAGAGAGAGAC	52	200-900	92	63	68.48
ISSR-2	CACACACACACACACAG	52	150-900	99	76	76.77
ISSR-3	GTGTGTGTGTGTGTGTC	52	250-700	48	25	52.08
ISSR-4	GAGAGAGAGAGACC	40	250-700	79	48	60.76
ISSR-5	CACACACACACAGG	52	200-900	52	48	92.31
ISSR-6	GAGAGAGAGAGAGG	40	150- 400	47	31	65.96
ISSR-7	TGTGTGTGTGTGTGG	40	200-700	50	32	64.00
ISSR-8	GAGAGAGAGAGACC	40	200-700	43	30	69.76
ISSR-9	GTGTGTGTGTGTCC	40	250-800	66	45	68.18
Total 9				576	398	69.10

TAF = Total amplified fragments, PB = Polymorphic bands and PB (%) = Percentage of polymorphism.

Total protein and SDS-PAGE

Total protein was extracted from 2 g fresh weight of plant leaves. Each sample were grinded with 10 mL of Extraction buffer (0.5M Tris-HCl (pH 6.8), 10% sucrose, 2% SDS, and 5% 2-mercaptoethanol). The slurry was centrifuged at 5000 rpm for 20 min. Three milliliter of ammonium sulphate solution were added 1 mL of the supernatant to precipitate the proteins then kept overnight in a refrigerator. It was then centrifuged at 5000 rpm for 20 min. the pellet was washed two or three times in 70% acetone. SDS-PAGE was performed by the method described by **Laemmli, (1970).** Protein was analyzed on 1.5 mm thick and 15 cm long gels run in a dual vertical slab unit (Hoefer Scientific Instruments, san Francisco, CA, USA, MODEL SE 600 Series Hoefer, Pharmacia Biotech). From each sample, 50 µl of extract was loaded a polyacrylamide gel. The separation gel (12%) and staking (4%) were prepared from an acrylamide monomer solution. Electrophoresis was carried out at constant current of 35 mA through the stacking gel, and at 90 mA through the separation gel at 4°C After electrophoresis the gel was stained by Coomassie Brilliant Blue R-250 and the molecular weight (MW) of protein corresponding to each band was calculated by protein marker with kilo Daltons (kDs) molecular weights.

Data analysis

The ISSR bands were scored using the binary scoring system that recorded the presence and absence of bands as 1 and 0, respectively for each relative position. Genetic similarity between a pair of faba bean cvs. was calculated using Nei and Li's index of similarity (**Nei and Li 1979**). Cluster analysis was conducted based on genetic similarity estimates using the unweighted pair-group method arithmetic average (UPGMA) procedure in NTSYS-pc version 2.1 software package (**Rohlf, 2000**) in order to deduce genetic relationships among various faba bean cvs.

3. Results and Discussion

ISSR analysis

The genetic diversity and genetic relationship among 10 faba bean cultivars collected from two different geographical locations in Egypt (Table 1) was evaluated, using ISSR assay. The nine ISSR primers amplified 576 bands, 398 bands out of them were polymorphic. The percentage of polymorphism of the amplified products was 69.1%. The size of all amplified bands ranged from about 150 to 900 bp (Table 2).

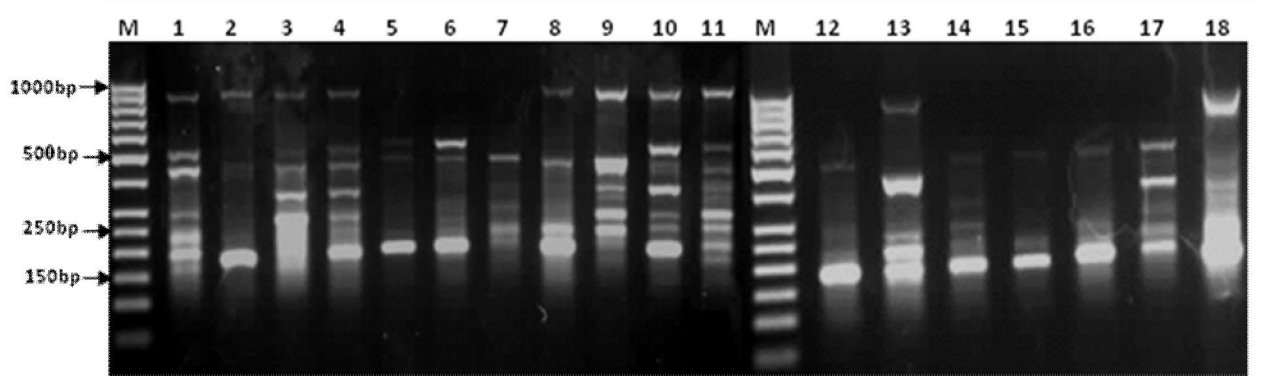
The average of the total amplified bands per studied primer was 64, ranging from 43 to 99 bands. For the polymorphic bands, the average was 44.2 amplified bands per primer, representing 69.1% of polymorphism. These results are relatively close with those of **Terzopoulosa and Bebeli, (2008)**, who found that the percentage of polymorphism revealed within the Greek faba bean populations ranged from 37.5% to 84.62% with an average of 67.48% using 11 ISSR primers.

The ISSR banding patterns of 10 faba bean populations, which were amplified by ISSR primers; ISSR-1,ISSR-2 and ISSR-5 are shown in Figs. (1 a, b and c).

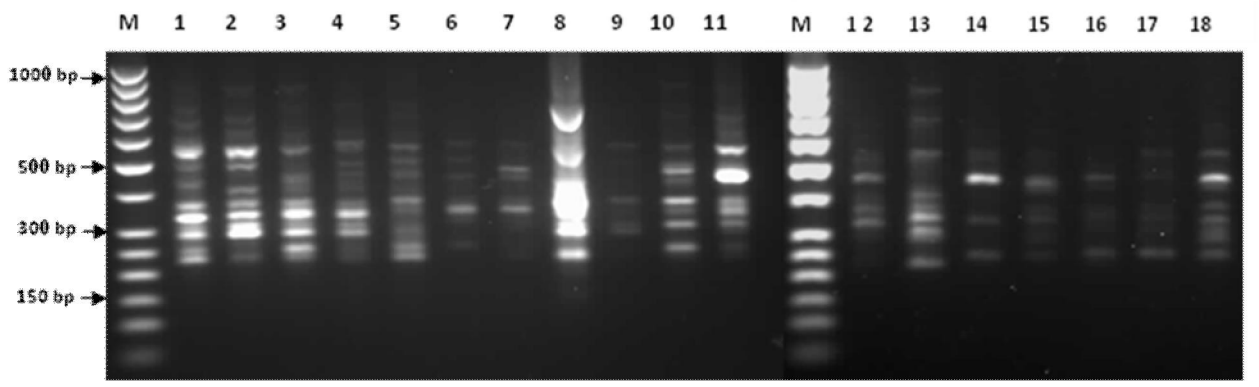
The genetic similarity among the 10 faba bean cvs. ranged from 24% to 95% (Table 3).

The highest genetic similarity was registered for two green large-seeded cvs. (Aspany1 and Aspany 2) as well as two dry small-seeded cvs. Sakha 3 and Rena Mora (95%) followed by ILB 450 and

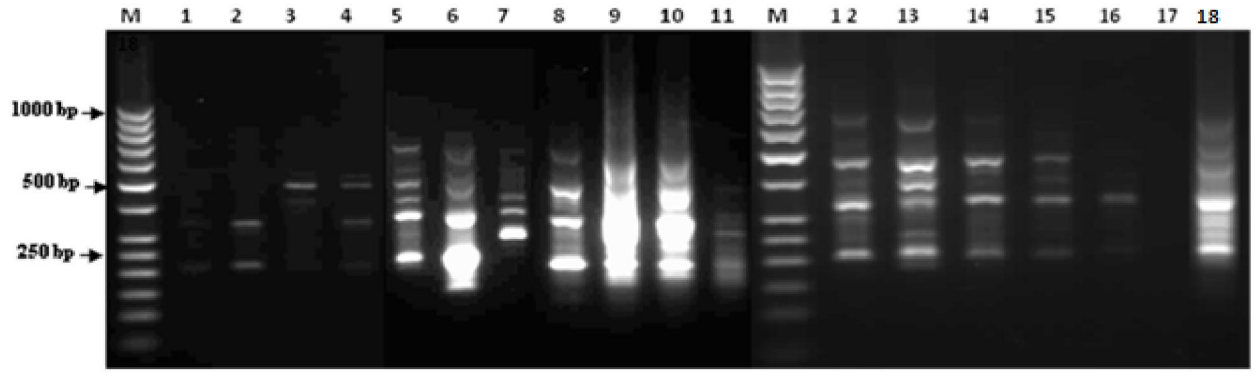
Giza 843 (94%). However, the lowest genetic similarity (24%) was observed in cvs. Sakha 3 and ILB 450 as well as green large-seeded Aquadolce and dry small-seeded Misr I cvs. (57%) followed by green large-seeded Aspany 1 and dry small-seeded Misr I (58%). Similar results were reported by **Duc *et al*. (2010)**, who found that the faba bean offered an enormous genetic variability for breeding purposes.



(a) Primer ISSR-1



(b) Primer ISSR-2



(c) Primer ISSR-5

Figure(1 a, b and c): Banding pattern of various faba bean cvs. generated by ISSR primers under two different geographical locations (Fuka and Abies). Numbers 1-18 stand for the faba bean cvs. listed in Table (1). M is a DNA molecular-size marker**.**

Table (3): Similarity matrix showing the relationship among various faba bean cvs. in the two different geographical locations in Egypt based on ISSR detection data.

Experimental location	Fuka (Matrouh Governorate)								Abies (Alexandria Governorate)									
F. bean cultivars	ILB 450	Mis I	Gi 843	Sak 3	Re Mo	Gi 3	ILB 312	Gi 716	Aqu	Asp 1	Asp 2	ILB 450	Mis I	Gi 843	Sak 3	Re Mo	Gi 3	ILB 312
ILB 450	100																	
Misr I	91	100																
Giza 843	**94**	89	100															
Sakha 3	88	89	92	100														
Rena Mo	83	82	87	85	100													
Giza 3	79	80	79	83	86	100												
ILB 312	77	76	75	79	88	88	100											
Giza 716	83	86	79	83	76	82	82	100										
Aquadolce	77	78	75	83	72	70	72	78	100									
Aspany1	72	75	70	76	69	69	69	75	91	100								
Aspany2	75	76	73	79	72	72	72	74	90	**95**	100							
ILB 450	76	77	78	**24**	83	81	77	71	63	62	67	100						
Misr I	70	69	72	68	75	73	73	67	**57**	**58**	63	86	100					
Giza 843	74	75	76	74	79	81	75	71	61	62	67	86	84	100				
Sakha 3	77	76	81	75	80	80	74	70	62	63	68	85	83	91	100			
Rena Mo	76	75	80	74	81	81	71	69	61	64	67	86	82	92	**95**	100		
Giza 3	77	74	83	75	80	76	70	66	66	69	74	79	77	83	90	89	100	
ILB 312	68	69	74	70	71	71	65	63	61	62	67	82	88	88	83	84	87	100

Cluster analysis based on Nei's genetic distances using the UPGMA method could place the 10 faba bean cvs. into two main clusters: i.e., A and B (Fig. 2). The dry small-seeded cvs. (field beans) which cultivated under two different geographical locations; Fuka and Abies were grouped in cluster A. While, the green large-seeded cvs. (broad beans) were grouped in cluster B. Cluster A was further divided into two sub-clusters: A1 and A2. Sub-cluster A1 was subsequently composed of 8 small-seeded cvs. which cultivated under Fuka region. While, sub-cluster A2 included the small-seeded cvs. that grown under Abies region. The results indicated that the 8 dry small-seeded cvs.; i.e., ILB 450, Misr I, Giza 843, Sakha 3, Rena Mora, Giza 3, ILB 312 and Giza 716 which were collected from these two different locations were joined in one cluster (A). That's might be due to the high genetic similarity among the same cvs., particularly the most of them are Egyptian varieties introduced from ICARDA **(Ghandorah and El-Shawaf, 1993).** Therefore, ISSR technique could divide the 10 examined faba bean cvs. depending on the genetic similarity among them.

SDS-PAGE protein analysis:

The protein banding patterns based on SDS-PAGE for the various faba bean cvs. grown under two different geographic locations Fuka and Abies are shown in Fig. (3). The total number of bands were 25, having molecular weights ranged from 42 KDa to 95 KDa. High number of bands ranged from 8 to 15 observed in the cvs. grown under clay soil conditions (Abies location). While the lowest number of bands is shown in faba bean cvs. grown under desert soil conditions (Fuka location). This result might be due to increasing soil fertility of Abies coupled with enhancement of N uptake under this region, which was associated with increasing protein content of faba bean cvs., comparing with the same cvs. but grown under the poor desert soil of Fuka region.

Data in Fig. (3) can concluded that, it was possible to differentiate the closely related cvs. of faba bean on the basis of gel protein analysis under wide-ranging environments. This inconsistency of protein profiles can supported by the findings of **Duc, (1997),** who observed that protein contents of faba bean were divergent depending on the genotypes and environmental conditions. Similarly, **Alghamdi, (2009),** mentioned that the total protein content of faba bean was varied greatly among genotypes and locations. Moreover, **Ghafoor and Arshad (2008**) observed a considerable amount of variation in pea based on SDS-PAGE analysis under different locations.

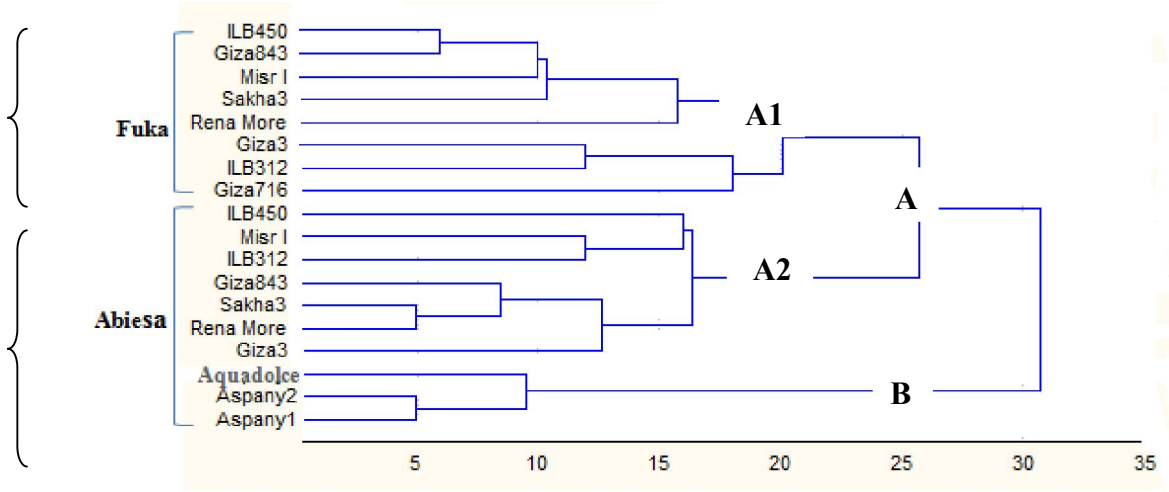


Figure (2): Dendrogram cluster analysis of various faba bean cvs. showing the genetic similarity anchored in ISSR-PCR primers data.

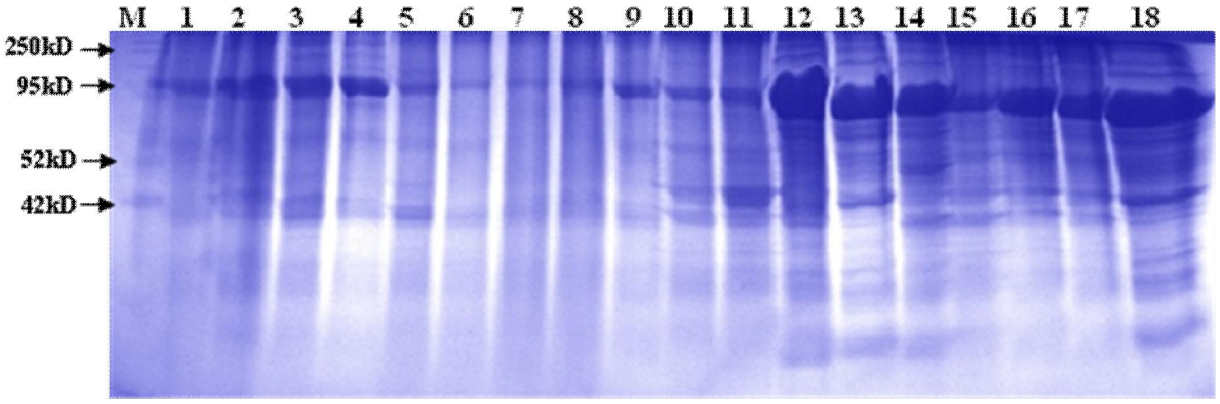


Figure (3): SDS-PAGE protein banding pattern of various faba bean cvs. grown under two different geographic locations (Fuka and Abies). M, referred to the protein standard marker with kilo Daltons (kDs) molecular weights.

Conclusion

ISSR markers are useful tool for detection the genetic diversity among *V. faba* cvs. and help in studying genetic relationships and clarifying taxa of the same species under two diverse geographic locations. Since, ISSR technique reflected enough polymorphism to distinguish among different dry small-seeded (field beans) and green large-seeded (broad beans). Also, it was possible to differentiate the closely related faba bean cvs. based on protein analysis.

References

1. Aguilera, J. G., L.A. Pessoni,G.B. Rodrigues, A.Y. Elsayed, D.J. da Silva, E.G.de Barros(2011):Genetic variability by ISSR markers in tomato (*Solanum lycopersicon* Mill). Revista Brasileira de Ciências Agrárias Recife 6(2): 243-252.
2. Ajebade, S. R., N.F.Weeden, S.M. Chite (2000): Inter simple sequence repeat analysis of genetic relationships in the genus *Vigna*. Euphytica 111(1): 47-55.
3. Alghamdi, S. S. (2009): Chemical composition of faba bean (*Vicia faba* L.) genotypes under various water regimes. Pakistan Journal of Nutrition 8(4): 477-482.
4. Bakry, B. A., T. A. Elewa, M. F. El karamany, M. S. Zeidan, M. M. Tawfik (2011): Effect of row spacing on yield and its components of some faba bean varieties under newly reclaimed sandy soil condition. World Journal of Agricultural Science 7(1): 68-72.
5. Belaj, A., Z.Satovic, G.Cipriani, L.Baldoni, R.Testolin, L. Rallo , I. Trujillo (2003): Comparative study of the discriminating capacity of RAPD, AFLP and SSR markers and of their effectiveness in establishing genetic relationships in olive. Theoretical and Applied Genetics 107: 736-744.
6. Chen, Y., R. Zhou, X. Lin, K. Wu; X. Qian, S. Huang (2008): ISSR analysis of genetic diversity in sacred lotus cultivars. Aquatic Botany 89: 311–316.

7. Duc, G. (1997): Faba bean (*Vicia faba* L). Field Crops Research 53: 99-109.
8. Duc, G; S. Bao, M. Baum; B.Redden, M. Sadiki, M. J Suso, M.Vishniakova, X. Zong (2010): Diversity maintenance and use of *Vicia faba* L. genetic resources. Field Crops Research 115: 270-278.
9. El-Danasoury, M. M., A. E. El-Ghubashy, H. Yossif , R. T. Behairy (2008). DNA fingerprinting to identify some faba bean (*Vicia faba* L.) varieties. Journal of Agricultural Science Mansoura University 33(6): 4619-4630.
10. FAO, (Food and Agriculture Organization). (2002): FAO Yearbook 2001: Production., vol. 55. FAO, Rome, Italy.
11. Galvan, M. Z., B. Bornet, P. A. Balatti, M. Branchard (2003): Inter simple sequence repeat (ISSR) markers as a tool for the assessment of both genetic diversity and gene pool origin in common bean (*Phaseolus vulgaris* L.). Euphytica 132: 297-301.
12. Ghafoor, A. , M. Arshad (2008). Seed protein profiling of *Pisum sativum* L. germplasm using sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) for investigation of biodiversity. Pakistan Journal of Botany 40: 2315-2321.
13. Ghandorah, M. O., I. S. El-Shawaf (1993): Genetic variability, heritability estimates, and predicted genetic advance for some characters in faba bean (*Vicia faba* L.). Journal of Kingdom of Saud University of Agricultural Science 5(2): 207-218.
14. Gilbert, J. E., R. V. Lewis, M. J. Wilkinson, P. D. S. Caligari (1999): Developing an appropriate strategy to assess genetic variability in plant germplasm collections. Theoretical and Applied Genetics 98: 1125-1131.
15. Gonzales, A., W. A. Delgado-Salinas, R Papa, P. Gepts (2005): Assessment of inter simple sequence repeat markers to differentiate sympatric wild and domesticated populations of common bean. Crop Science 45: 606-615.
16. Hu, J., M. Nakatani, A. Lalusin, T. Kuranouchi, T. Fujimura (2003): Genetic analysis of sweet potato and wild relatives using inter-simple sequence repeats (ISSRs). Breeding Science 53: 297-304.
17. Jabbarzadeh, Z., M. Khosh-khui, H. Salehi , A. Saberivand (2010): Inter simple sequence repeat (ISSR) markers as reproducible and specific tools for genetic diversity analysis of rose species. African Journal of Biotechnology 9(37): 6091-6095.
18. Khalafallah, A. A., K. M. Tawfik, Z. A. Abd El-Gawad (2008). Tolerance of seven faba bean varieties to drought and salt stresses. Research Journal of Agriculture & Biological Science 4(2): 175-186.
19. Laemmli, U. K. (1970): Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227(5259): 680-685.
20. Link, W., C. Dixkens, M. Singh, M. Schwall , A. Melchinger (1995): Genetic diversity in European and Mediterranean faba bean germplasm revealed by RAPD markers. Theoretical and Applied Genetics 90: 27-32.
21. Nei, M. , W. H. Li (1979): Mathematical models for studying genetic variation in terms of restriction endonucleases. Proceeding National Academy of Sciences of USA 76: 5269-5273.
22. Reddy, M. P., N. Sarla , E. A. Siddiq (2002). Inter-simple sequence repeat (ISSR) polymorphism and its application in plant breeding. Euphytica 128: 9-12.
23. Rohlf, F. (2000): NTSYSpc. Numerical Taxonomy and Multivariate Analysis System-Version 2.10b. Applied Biostatistics Inc, New York.
24. Rubatzky, V. E. , M. Yamaguchi (1997). World vegetables, principles, production, and nutritive values. 2nd edition. Chapman & Hall, ITP, New York. pp. 843.
25. Sudupak, M. A. (2004): Inter- and intra-species inter simple sequence repeat (ISSR) variation in the genus *Cicer*. Euphytica 135: 229-238.
26. Terzopoulosa, P. J. , P. J Bebeli (2008):Genetic diversity analysis of Mediterranean faba bean (*Vicia faba* L.) with ISSR markers. Field Crops Research 108: 39-44.
27. Vishwanath, K., K. P. Prasanna, H. M. Pallvi, P. S. Rajendra, S. Ramegowda, P. J. Devaraju, T. V. Anantharayanan (2011): Identification of tomato (*Lycopersicon esculentum*) varieties through total soluble seed proteins. Research Journal of Agricultural Science 2(1): 8-12.
28. Zied, M., C. C. Schon, W. Link (2003): Genetic diversity in recent elite faba bean lines using AFLP markers. Theoretical and Applied Genetics 107: 1304-1314.
29. Zeid, M., S. Mitchell, W. Link, M. Carter, A. Nawar, T. Fulton, S. Kresovich (2009): Simple sequence repeats (SSRs) in faba bean: new loci from Orbanche-resistant cultivar 'Giza 402'. Plant breeding 128(2): 149-155.

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